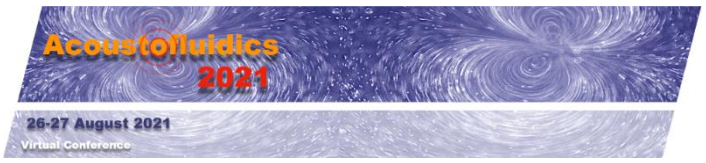


# Measuring cell density in an acoustofluidic micro-cavity

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## Introduction

In this work, we proposed a weighing scale for weighing cells (or any microparticle) based on a methodology combining sedimentation theory and computational imaging processing. First, the particles under investigation were levitated to a particular height (matching with the half-wavelength of the acoustic ~~wave produced~~) in the acoustic resonator center. The net force leading to the lift of the particles are mainly due to the acoustic force produced within the resonator filled with a proper fluid. Subsequently, the acoustic force is turned off, and the investigated particle is allowed to "free falling". The dynamics motion of the particle is then used to determine the density experimentally using a theoretical model proposed. As a first step, commercial polystyrene bead was used to calibrate the methodology since its density is well-known (~1050 kg/cm$^3$). After verifying the methodology, the density of single alive murine macrophages J774 was determined by using the acoustic weighing scale and the value was experimentally estimated to be 1040 +- 20.8 kg/m$^3$. The studied is a first step for biosensing, since the determination of the cell density is a fundamental parameter to monitor the physiological state of a cell

## Experimental setup and methodology for weighing cells

Fig. 1 schematically describes the acoustofluidic micro-cavity proposed for measuring cell density. The acoustofluidic device plays the role of a weighing scale while the confocal apparatus (composed by a set of lens and a CCD camera) is used to record images for calibrations and monitoring the dynamic motion (Fig. 1 (a)). The set of components forming the micro-acoustofluidic device is depicted in Fig. 1(b), composed by a piezoelectric transducer substrate; resin material and a thin coverslip reflector (150 μm of thickness). The lead zirconate titanate (PZT) was used as the piezoelectric material and acting to generate the acoustic waves that produces an acoustic radiation force to levitate any particle inside the device. The acoustofluidic micro-cavity is sealed to enable the establishment of standing acoustic waves within it, creating an acoustic pressure distribution and well-localized pressure node and antinodes in the cavity. This picture is well-observed in the illustration of Fig 1c-d, before applying voltage (~ 4V) there is no establishment of acoustic pressure within the acoustofluidic cavity (Fig 1c), but as the voltage supply is turn on it is clearly observed an acoustic pressure gradient in the cavity (Fig 1d), which in turn induce an acoustic radiation force that propels a dynamic motion on any microparticles inside the acoustofluidic cavity and handling it to be levitated at the pressure field node as pictured in the Fig. 1(d). The weighing scale methodology is based on sedimentation motion (sedimentation theory.[1,2]) of the sub-micron particle investigated. Two scenarios is reported here, polystyrene single-bead and single-macrophage cell (live murine J774). The dynamic motion of the scenarios (position vs time) investigated is monitored during the falling state after the acoustic waves been switched off (see Fig. 2). The forces acting on the systems is illustrated in (e). Calibration curves of position dependence (in and out of the light focus) of relative area of defocusing of the two scenarios of polystyrene single-bead and single-macrophage cell (live murine J774) are displayed in Fig. 1(f).

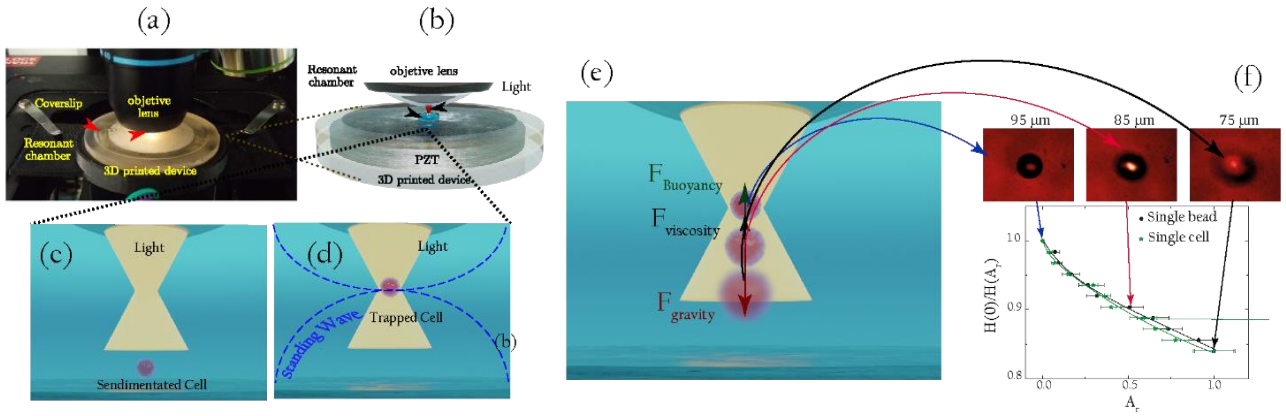


**Figure 1:** (a) Photography of the acoustofluidic micro-cavity proposed for measuring cell density. The system is composed by an acoustofluidic deviceand a confocal apparatus. (b) Micro-acoustofluidic device is composed by a piezoelectric transducer substrate (acting as a transducer to excite acoustic vibrations), a resin material (providing a high acoustic impedance) and a reflector glass (to seal cavity). In our studies the solution was made with polystyrene microparticles dispersed in destillated water and PBS. (c) A sub-micron spheres are laid down on the bottom of the acoustic cavity. For this case, the power to induce acoustic wave is off. (d) As the power to promote acoustic wave is on, the submicron-spheres can be levitated and trapped in a plane node promoted by standing acoustic waves in the cavity. Calibration curves of position dependence of relative area of defocusing of the two scenarios of polysterene single-bead and macrophage single-cell (alive murine J774) are displayed in (f). The dynamic motion of the scenarios (position vs time) investigated is monitored during the falling state after the acoustic waves been switched off. The forces acting on the sytems is illustrated in (e).

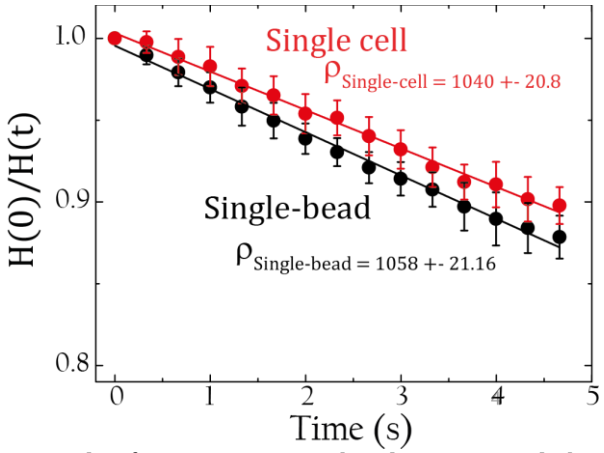


**Figure 2:** Falling dynamics of a particle of two scenarios such polysterene single-bead (black dots) and macrophage single-cell (alive murine J774, red dots). Position versus time is monitored in 'real' time. The density of the macrophage single-cell was determined to be 1040 +- 20 kg/m$^3$. For each scenario, 5 measurements was performed and the data are the associated average values

## Dynamic motion during falling state

The falling distance as a function of time of both scenarios investigated is obtain experimentally after allowing the particles falling down under the action of buoyance, viscosity and gravitational force (see Fig. 2). A theoretical model was proposed based on the sedimentation theory in the regime of h>> r,[1,2] where h is the distance from the bottom of the sub-micron particle to the bottom of the device.[1,2]

## Conclusion

The density of the commercial polystyrene single-bead was experimentally determined to be 1058 +- 21 kg/m$^3$, matching with the value provided by the fabricante ( ~ 1050 kg/m$^3$). No necessary correction was employed in the theoretical model used. Using the same methodology and model, the density of the macrophage single-cell (alive murine J774, red dots) was determined to be 1040 +- 20 kg/m$^3$. We are looking forward to presenting our results in the international acoustofluidics community at *Acoustofluidics 2021 Virtual Conference* on 26 - 27 August 2021.

**References** (Times New Roman 10 pt)

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